

Fluorescence measurement using Varioskan LUX

In fluorescence intensity (FI) or time-resolved fluorescence (TRF) measurements, the following actions are carried out by the instrument:

1. The tray is driven in.
2. In fluorescence intensity measurements, excitation and emission wavelengths are selected by rotating the excitation and emission monochromatic gratings. On the other hand, in time-resolved fluorescence measurements the emission wavelengths are selected by rotating the filter wheel.
3. In the signal level calibration procedure the instrument reads the fluorescence from the reference chip, compares it to the value in non-volatile memory and sets a factor to correct the reading. In long measurement procedures calibration is performed in a suitable phase without disturbing the measurement timing. The default calibration interval is 10 minutes.

4. The instrument uses the dynamic range setting the user has selected in the SkanIt Software measurement session :

- AutoRange
- Manual range
- High range
- Medium high range
- Medium low range
- Low range

The measured values are comparable regardless of the dynamic range selection, AutoRange or any of the fixed manual ranges.

5. The wells are measured with a selected measurement time that can vary from 10 to 1000 ms in fluorescence intensity measurements and from 10 to 10 000 ms in timeresolved fluorescence (TRF) measurements. It is recommended to measure using a 100 ms measurement time in fluorescence intensity

measurements and 1000 ms in TRF measurements, which normally produces good results. If it is necessary to improve the quality of the results, the flash amount should be increased. The result is the mean value of individual 10 ms readings during the total measurement time. With TRF measurements there are two additional user-defined measurement parameters:

TRF delay time and TRF integration time. The TRF delay time defines the time difference between the excitation flash and the start of emission signal collection, while the TRF integration time defines the time used for emission signal collection. When the Varioskan LUX performs a TRF measurement, it excites the sample with a very short light pulse, waits for the defined TRF delay time and then collects the signal during the defined TRF integration time. These actions

form one TRF measurement cycle (Figure 4–38), which is performed within a 10 ms period. The cycle is repeated as many times as defined by the measurement time.

6. If necessary, set the settle delay in SkanIt Software. For more details, refer to “Settle delay” on page 52.